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Canine Parvo Virus: A Review on Current Perspectives in Seroprevalence, Diagnostics and Therapeutics

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Abstract: An enteric virus named Canine parvovirus type 2 (CPV-2) has become the most important wild and domestic canine pathogen since it was first reported in 1978. The virus exhibited seroprevalence across all continents. In just a matter of few decades, genetic mutations have led to the emergence of three antigenic variants referred to as CPV-2a, CPV-2b and CPV-2c. Antigenic changes have also correlated with the broadening of host range with subsequent propagation in wild carnivores. Canine parvovirus type 2 (CPV-2) has been associated with extremely high morbidity and mortality rates in younger dogs. Several molecular and serological tests may be employed for the diagnosis of CPV disease. In Pakistan multivalent live attenuated viral vaccines have been made available for canine immunization. However, control and prevention from this disease has become even more convoluted as newer variants have emerged which can involve domestic feline and wildlife carnivores. A whole slew of DNA, peptide and recombinant vaccines have been under development to counter this risk of a sudden outbreak. In precis, mass vaccinations of stray, pet and wild canine populations along with implementation of strict disinfection protocols at kennels could help control spread of disease.

Key words: Antigenic Variants · Canine Parvovirus Type 2 · Hemorrhagic Enteritis · Immunization · Seroprevalence

INTRODUCTION

One of the most important pathogens in dogs is Canine parvovirus type 2 which has been proven to cause myocarditis and hemorrhagic enteritis in young dogs. The causative agent was first discovered in 1977 since then it has been attributed with high morbidity and mortality rates throughout the world [1]. Canine parvovirus (CPV) is supposed be a mutated strain with a different host range of feline panleukopenia virus (FPV) that instigated its spread into domesticated canine populations from feral carnivores namely foxes and minks. This particular disease is identifiable either by severe intestinal hemorrhage along with diarrhea and vomiting or by myocarditis with ensuing infarction in younger pups [2]. In recent years highly pathogenic strains of CPV-2 viral infections are being reported in canine populations. It is a highly contagious and transmissible virus. In Pakistan due to a large pool of unvaccinated stray canine population risk of disease and death amongst household pet due to CPV has been deemed critical. Vaccine failures are a point of contention amongst the veterinary community as some relate it to maternal antibodies while other believe it to be a consequence of point mutations

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and variations in viral genome [3]. However, in the last decade or so serological and molecular techniques have developed to such an extent for effective diagnosis of the disease that early identification and control have become quite possible. Therefore, present review on Canine Parvo diseases is intended to deliver comprehensive knowledge regarding immune-prophylaxis, diagnosis and treatment to researchers, veterinary practitioners, pet owners and students [4].

Canine Parvo Viral Structure: Canine parvovirus (CPV) is a single-stranded DNA virus identifiable by its small size of 5.2 Kb under an electron microscope belongs to genus Parvovirus and family Parvoviridae [5]. Molecular weight (MW) of virus is 5.5 to 6.2 9 10^6 Da while its buoyant density in cesium chloride (CsCl) is 1.39-1.42 g/cm^3. Structurally, the virus is icosahedral and it has two promoters responsible for the expression of three structural (VP1, VP2 and VP3) and two non-structural proteins (NS1 and NS2). Both VP1 and VP2 are essential components of viral capsid while VP3 is a post-translational product of VP2 protein expression obtained after enzymatic cleavage by trypsin which is only found in complete Viruses [6]. On basis of protein subunits primordial capsid organization in CPV-2 have been found comparable 5-6 subunits of VP1 and 54-55 subunits of VP2 [7]. The organizational motif of VP1 is eight stranded where by one-third of nucleotide base pairs are present in the antiparallel b-barrel form [6] while the remaining two-thirds are oriented as loops. Information pertaining to host receptor binding and antigenic expression in tissues is primarily mapped onto the loops linking b-barrel strands. Viral replication takes place in the nucleus of the progenerating cells leading to observable intranuclear inclusion bodies. Icosahedral capsid with 15 A° canyon-like depression is representative features of parvovirus [8].

Canine Parvovirus Variability: By the end of 1960s a new infectious disease was being reported in pups characterized by high mortality rates due to either severe gastroenteritis or myocarditis. Fecal examination of infected patients revealed presence of a small, non-enveloped virus under electron microscope. This contagion promptly named as CPV-2 was later isolated in both canine and feline tissue cultures.

CPV Variants in Pets: Since its emergence it has been hypothesized that virus evolved as a result of subsequent mutations that accumulated during a prolonged period of time until it finally found a favorable host in Dog [9]. These deductions have been corroborated in several studies where virus underwent several mutations in canine tissue cultures during repeated passages [10]. Phylogenetic analysis has also indicated common ancestry amongst all CPV variants that emerged during the mid-1970s and feline panleukopenia virus (FPV). The comparison in VP2 genes of canine parvo virus and feline panleukopenia revealed that only six coding nucleotides at gene positions 3025, 3065, 3094, 3753, 4477 and 4498 were different in both viruses. It was owing to such mutations that CPV-2 acquired the ability to propagate in canine cells [11]. A CPV-2 variant identifiable as CPV type 2a became endemic all over the world within a year due to antigenic drift in sequence of capsid genome by replacing Met to Leu at 87, Ala to Gly on 300 and Asp to Tyr at 305 of canine parvo virus VP2 residues. Moreover, another antigenic variant namely CPV type 2b which was discovered in 1984, was found to differ at 555 from Ile to Val and 426 from Asn to Asp [12]. CPV type 2a and 2b both are endemic in varying ratios across different canine populations. Recently a third variant namely CPV-2c has also emerged in dogs from some south east Asian and European countries [13, 14]. Differentiation amongst these variants is not possible based on their clinical signs however monoclonal antibodies have been developed for their detection [15] (Fig. 1).

CPV Variants in Wild Animals: Some of the Scandinavian countries reported the spread of the virus in wild and feral populations of canine species in 1976. Coyotes have been observed with clinical manifestation of the disease which was verified by isolation of VP2 gene through DNA sequencing. However, It has been reported that Raccoons could tolerate clinical manifestation of CPV-2 infection [16]. Similarly, clinical infection and serological prevalence of the aforementioned affliction have been reported in jackals, grey foxes and other kinds of prairie dogs [17]. Lately, certain reports have emerged that have asserted discovering CPV-2a and CPV-2b DNA in certain wild felids as well. This phenomenon may be rationalized by postulating a higher susceptibility of wild felids as opposed to domestic ones for CPV-2a/ 2b infections [18].

Geographical Distribution of CPV Variants Around the Globe: Infections of canine parvo virus have been reported in a wide variety of canine species and incidence of the disease has been observably high in instances where a large number of dogs are housed in close vicinity.

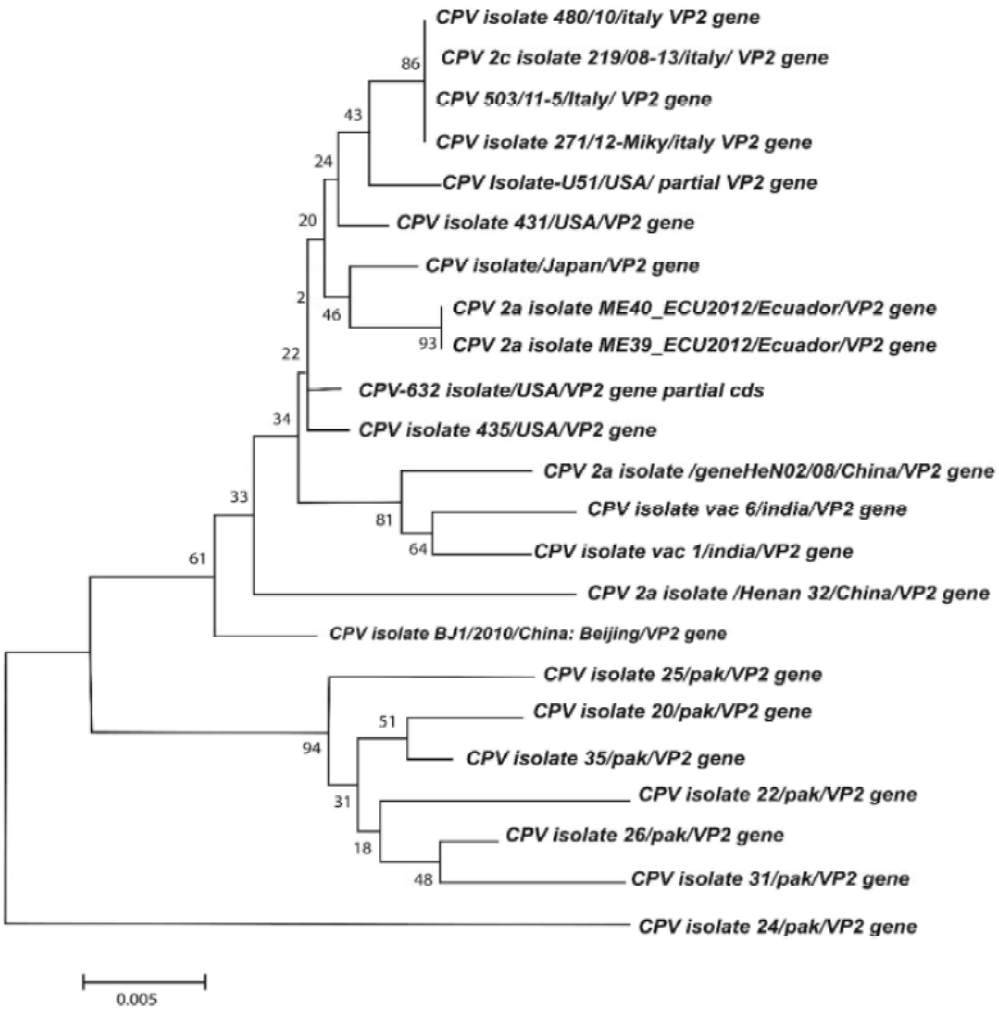

Fig. 1: Phylogenetic tree of partial VP2 nucleotide sequences of canine parvovirus strains obtained from the GenBank database and Pakistani CPV strains [93]

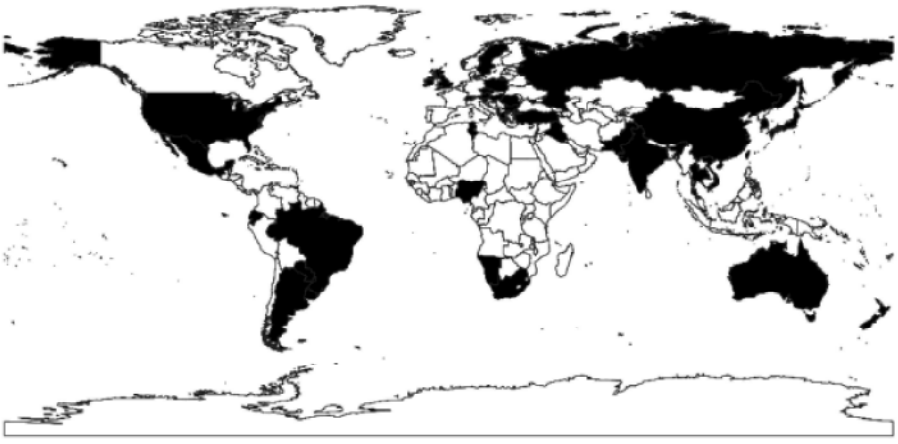

Fig. 2: Seroprevalence of CPV-2 variants in domestic dogs around the world (Endemic disease regions are highlighted in darker color)

Table 1: Distribution of CPV-2 variants around the world

Region	Country	CPV-2 variants detected		
		CPV-2a	CPV-2b	CPV-2b
Europe	UK [77]	18	21	1
	Ireland [78]	3	4	0
	France[79]	4	2	1
	Belgium[77]	1	1	0
	Austria [80]	*	*	0
	Germany [79]	5	2	1
	Spain [81]	3	1	9
	Portugal [82]	2	94	102
	Italy [81]	13	3	5
	Greece [83]	81	1	2
	Poland [84]	11	2	19
	Switzerland [80]	**	**	0
	Sweden [85]	0	0	4
	Slovenia [86]	1	0	0
	Bulgaria [87]	171	40	5
	Romania [86]	2	0	0
	Hungary [88]	24	0	0
	Albania [89]	29	0	24
	Czech Republic [77]	1	0	0
Central Asia	Turkey [90]	17	8	0
	Russia [91]		***	
Middle East	Iraq [92]	3	6	0
Subcontinent	India [16]	27	39	12
	Pakistan [93]	6	0	0
South east Asia	Vietnam [15]	0	7	4
	Thailand [94]	19	7	0
East Asia	Taiwan [95]	35	19	0
	China [96]	20	2	0
	South Korea [97]	41	3	0
	Japan [98]	9	95	0
Southern Africa	South Africa [99]	6	13	0
	Namibia [100]	3	9	0
Northern Africa	Nigeria [99]	6	0	0
	Tunisia [101]	15	21	14
Oceania	Australia [102]	41	1	0
	New Zealand [103]	69	0	0
North America	USA [104]	1	19	7
South America	Mexico [105]	0	0	5
	Brazil [106]	1	8	33
	Uruguay [107]	20	0	130
	Argentina [108]	2	3	50
	Paraguay [108]	0	0	1
	Ecuador [109]	2	22	29

NA means that data is not available.

35 samples from Austria (*) while 14 from Switzerland (**) yielded new antigenic types CPV-2a and CPV-2b.

(***), information regarding Russia was obtained from GenBank resources i.e., accession number JN033694

In puppies under the age of 4 months, CPV has proven to be quite devastating however virus has been reported in dogs of all ages. Inbred breeds such as German Shepherd, English Springer Spaniels and Rottweilers are highly susceptible while crossbreds resist the severe clinical phase of disease [19]. Virus cannot be transmitted to humans and most researchers believe that after infection dogs are immunized for life against this virus [20]. Distribution of CPV and its various strains is sporadic and, in several countries (Fig. 2).

CPV-2a has been found endemic in Italy, France and Taiwan while CPV-2b has been reported in countries like South Africa [21], Japan [22], Brazil [23], Switzerland [24], USA [21]. Moreover, both these strains are believed to be equally distributed in UK[22] and Spain [25]. Researchers have reported third variant of canine parvovirus (CPV-2c) in North America [21], Spain [25], Vietnam [26], South America [27] and United Kingdom [28] (Table 1).

In Pakistan, Both CPV type 2a and 2b have been reported using strain specific primers. Despite modified live vaccine being used to vaccinate domestically kept dogs, it is not uncommon to still observe incidence of disease in pups [29–31]. Molecular and phylogenetic analysis of viral specimens have revealed that the most prevalent strain appears to be CPV-2a with elevated genetic heterogeneity in nucleotide and codon sequence [32]

Transmission: Canine parvovirus has been proven to be contagious and spreads by contact with canine infected feces or contaminated surfaces whereby the virus enters the body through oral route [33]. Dogs kept together in large numbers are at a higher risk of disease than the ones who are kept indoors and not allowed to be in contact with other dogs. The virus is quite resistant to ambient temperature and desiccation thereby allowing it to survive in ground tainted with feces for about 5 months [34] (Fig. 3).

Pathogenesis: As skin of the animal comes into contact with feces of clinically infected dogs, the hair and skin pick up the contagion. As these dogs' groom themselves the contaminant enters their gastrointestinal tract by oral route. Disease incubation time ranges between 3 to 7 days followed by period of illness. As the virus enters the host it undergoes multiplication in its lymphatic system [35]. Virus continues to enter the blood stream during replicative phase causing viremia and during the course of next few days it finally reaches organs with high rate of cellular proliferation. After reaching the hemopoietic

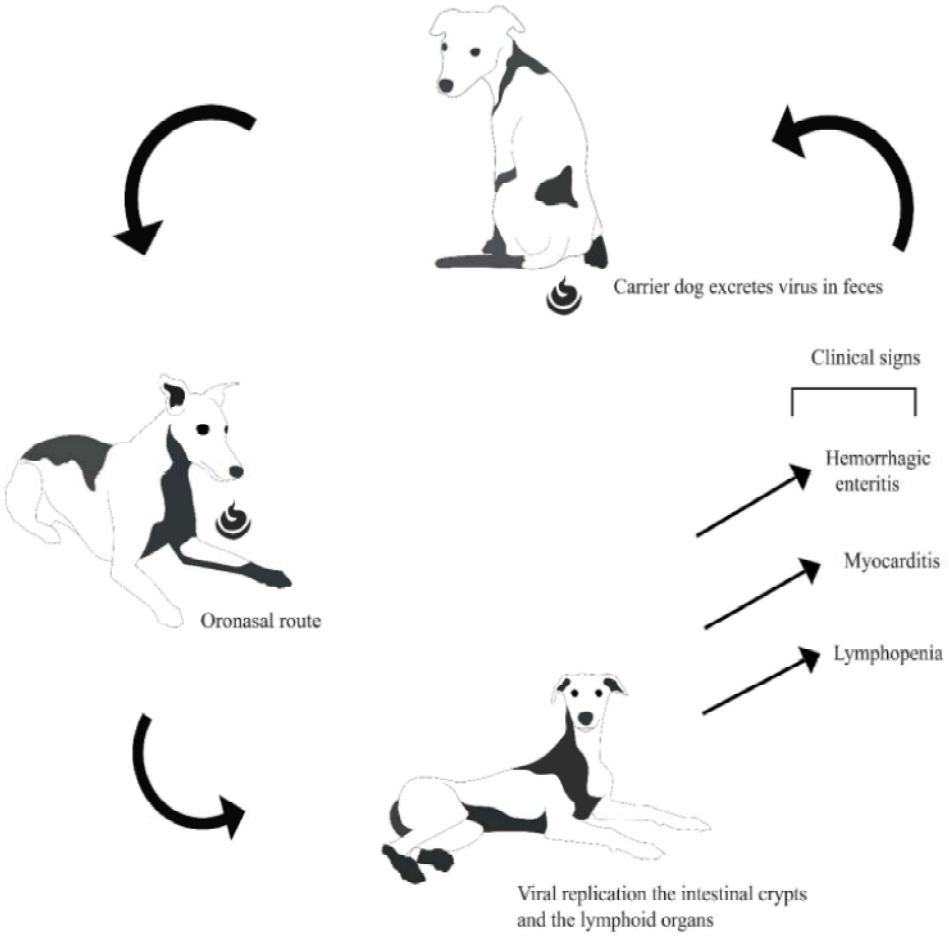

Fig. 3: Transmission of Canine Parvovirus in domestic dogs

system residing inside bone marrow canine parvo virus destroys the leukocytic proliferation thereby leading to leukocytopenia causing impairment of dog's immune defense mechanism [36]. However, it's the Gastrointestinal tract that's most heavily damaged due to infection. Virus destroys the "villi" and "microvilli" structure by dividing at Crypts of Lieberkuhn thereby compromising their ability to replace cells at the villi to maintain adequate assimilatory barrier and surface area [37]. Impairment of the vascular barrier and constant cellular sloughing at villi causes bloody diarrhea and may lead to secondary bacterial infections as intestinal bacteria continue to enter the blood stream. Death in most cases is attributed to persistent vomiting and diarrhea leading to severe dehydration followed by hypovolemic shock.

Clinical Symptoms: Canine parvo virus is deadly in unvaccinated young pups. Diarrhea is a hallmark indication of this disease however the severity is amplified in younger dogs. In early phases of the disease dogs experience inappetence, depression, pyrexia, vomiting and diarrhea. As the animal continues to lose fluids, body temperature gradually deprecates to subnormal levels [38]. Feces appear watery and may become sanguineous in later stages of the disease. Progression and pathogenesis of disease following 3rd day of clinical elucidation is highly variable due differences in viral exposure. Resistance to this infection is predicated upon age of the animals, exposure to risk factors and disease prevalence. Severe dehydration as a consequence of diarrhea and vomiting has in some instances, killed puppies as early as 2 days after manifestation of symptoms. Canine parvovirus can also manifest itself clinically as cardiac myopathy and myocarditis in puppies younger than three months [39]. It is commonly observed that in such cases 2/3rd of litter dies within 8 weeks of age while the survivors suffer cardiac insufficiencies for the remainder of their life [40]. Pup afflicted in such a manner my become ischemic and dyspneic. Younger pups

aging between 4 to 8 weeks suffer from acute heart failure while older pups typically 8 weeks experience subacute heart failures [36]. Serosanguinous ascites along with hepatomegaly are frequently observed [41]. Animals exhibiting cardiac signs don't have diarrhea, as virus proliferates in muscles of heart as opposed to intestinal villi.

Pathological Changes: Cytopathic and pathological lesions vary depending upon the type and severity of disease manifestation. In enteric form of the disease segmental sub-serosal lesions are usually observed in jejunum and ileum [42]. The lymphatics in mesentery are edematous and cross-sectional petechial hemorrhages are observable during acute stage of the disease [39, 42]. Cardio-myopathic form of the disease is discernable by visible cardiomegaly and myocarditis with distension of left atrium and ventricle. Pulmonary edema with frothy fluid in interstitial parenchyma do not allow lungs to collapse. Ascites with variable degree hepatomegaly due to passive congestion of portal circulation are also observed. white streaks often associated with cellular infiltrate are also present in the ventricular myocardium [43].

Viral multiplication is often confined to areas of high cellular regeneration therefore necrotic lesions in enteric crypts during early phases of the disease are quite common [44]. Sometimes, extensive epithelial loss significantly reduces the absorptive surface area and may lead to death due to endotoxic shock or electrolyte imbalance. Despite the pernicious nature of infection, as mitotic proliferation continues hyperplastic epithelial cells are observed in the mucosae. Whereas, Peyer's patches and thymus in young dogs become necrotic [45].

Diagnostic Techniques: Clinical symptoms, namely smelly diarrhea, vomiting, lethargy, inappetence and fever can be used to tentatively identify a clinical case of CPV infection. However, several serological tests can be employed for confirmatory diagnosis. Moreover, viral isolation on Madin-Darby Canine Kidney (MDCK), Cellosaurus A-72 and Crandell-Rees Feline Kidney Cell (CRFK) or identification through electron microscopy may also be a viable confirmation strategy [46]. DNA amplification techniques such as loop-mediated isothermal amplification (LAMP) [47] and real time PCR [48] are also used to determine seroprevalence and identify serotype from small viral DNA sample.

Hemagglutination (HA) Assay: HA has been successfully performed using swine, feline and primate red blood cells. The viability of HA for the diagnosis of fecal CPV shedding cannot be denied either. During the first week of post infection period titer has been reported to range between 128-10, 240. But the sensitivity of test fails by the second week post infection [32]. The limited sensitivity of Hemagglutination (HA) Assay has become a severe bottleneck in its varied use. However, specificity can be improved by treating fecal samples with $CHCl_3$ (10% V/V) or fluorocarbon (Genetron, Freon 113) [49]. The required amount of freshly washed RBCs and their relatively poor reactivity to antigen has limited its usage [50]. Best results for HA test have been observed after plates have been refrigerated at 4C and pH was maintained between 4 and 6 by using Phosphate buffer saline [51].

Enzyme Linked Immunosorbent Assay (ELISA): The functioning of this test is predicated upon antigenic reaction with specified monoclonal antibodies attached on to nonreactive containers or substrates [52]. This type of test is effective, reliable and can be performed at veterinary clinical facilities. ELISA testing has been employed to detect viral antigen of CPV from blood serum and fecal matter. Moreover, due to immense sensitivity and versatility sandwich ELISA has been routinely used for the detection of CPV antigen. The effectiveness of this diagnostic test is truly recognized in cases of parvovirus identification in puppies [53, 54].

Electron Microscopy: Electron microscopy can be effectively employed for the identification of CPV. Virions may be observed readily in feces by using negative staining. However, the specificity of this technique is poor due to the structural similarities between FPV and CPV [55].

Isolation and Culturing of Canine Parvovirus: Canine Parvovirus virus has been isolated in samples obtained from acute incidences of myocarditis or enteritis caused by CPV which can subsequently can be cultured on cell lines like CRFK (Crandell Feline Kidney) or MDCK (Madin-Darby Canine Kidney). Howbeit, specialized cell cultures, namely canine cell line (A-72) have especially helped researchers characterize CPV on biochemical and molecular basis. The highly fibroblastic characteristics of this cell line can visualize cytopathic effects produced by virus for up to 135 passages. Despite the diagnostic potential of this cell line the neoplastic origins of this drug make it a poor substitute for vaccine production [56, 57].

Polymerase Chain Reaction (PCR): PCR has been frequently used for confirmatory diagnosis of CPV [58].

The highly specific and sensitive nature of this test has made it a dependable method for disease identification [58]. Moreover, the ability of this test in differentiating various strains as well as serotypes of CPV-2 makes it an extremely important technique from research perspective [48]. Nested PCR is being employed these days to improve upon the sensitivity of this test even further. The improvement in diagnostic capabilities is evident from its ability to detect (RF) DNA when present in as low as 100ag concentration[59]. The immense strides that researchers have made in serotyping different strains of CPV have been accomplished through performing PCR of infected samples subsequently followed by Restriction fragment length polymorphism (RFLP) and sequencing. The differentiation between CPV2a and CPV2b was undertaken by digestion of viral genome (VP1/VP2 gene) using restriction enzymes namely Hpa1 and Rsa1 after its amplification. Moreover, it was reported that further enzymatic digestion of amplicon by using Alu1 could distinguish CPV-2a from CPV-2b [60]. Whereas CPV-2c was identified by fragmentation of PCR products by employing MboII. But research has shown that unambiguous differentiation of these viral strains requires using a TaqMan assay with minor groove binder (MGB) probe technology. Such a technique has been termed as Real time PCR (RT-PCR) [61]. MGB probes have been proven quite effective in elucidating polymorphism of capsid protein within different variants of CPV. SYBR Green I (SG) based RT-PCR has been quantifying CPV-2 variants in fecal samples of dogs [5]. The major benefit of real time PCR is in the fact that agarose gel electrophoresis has been made redundant as images can directly be processed on the monitor.

Loop-Mediated Isothermal Amplification (LAMP): Technique used for DNA detection is called as Loop Mediated Isothermal Amplification of DNA (LAMP). Whereby in case of CPV-2, VP2 gene targeting primers are used to amplify certain genes in DNA. This test was determined to be highly sensitive and relatively specific with 76.9% accuracy. An extremely small amount of genetic material i.e. 10^{-1} median tissue culture infective doses ($TCID_{50}$)/ml could be detected by this method [61].

Nucleic Acid Hybridization/Dot Blot: Firstly, fecal samples from suspected animals are inoculated on to cell culture. DNA is extracted from the supernatant of this growth medium. Extracted material is subsequently charged onto nylon membrane or nitrocellulose paper followed by hybridization using a radio-labelled probe [62].

Canine Parvovirus Detection by In-Situ Hybridization: CPV-specific nucleic acids were identified using CPV-specific DNA probe encoded with VP-1 and VP-2 capsid proteins indicating CPV specific nucleic acid distribution in infected tissue samples [62].

Nucleic Acid Sequencing: After amplification of genomic sample an automated DNA sequencer is used to type CPV strains with the help of appropriate primers. Computational analysis of this data provides researchers with phylogenetic analysis of the isolates [41].

Immunization: Colostrum is the natural method for immunizing young pups against viral threats. Researchers have judiciously performed serum neutralizing antibody tests to evaluate antibody titers capable of producing effective immunity against infection. The HI titer, 1:80 or more was estimated to be adequately protective against CPV-2 in dogs [63].

Puppies birthed by immunized bitches acquire protection against the infection from colostrum. These antibodies prevent incidence of disease for the first week of puppy's life. However, infection rates seem to spike in older than 6 weeks puppies as maternal antibodies continue to wane off 2–3 days after birth. A predominant percentage of pups only develop appropriate antibody titers if they are vaccinated after 12^{th} week of birth [64, 65]. Consequently, there is a duration of time when puppy is devoid of immunization and maternal antibody protection which is thereby called critical period.

Live Attenuated Vaccines: More often than not, multivalent vaccines are used to immunize young dogs which contain antigens namely; Canine parvovirus, Canine distemper virus, Parainfluenza virus, Leptospira bacterin and inactivated rabies virus [66]. But most clinicians practicing in highly endemic regions prefer using a monovalent CPV-2 for immunizing younger pups which contains very high titer virus (10^7 $TCID_{50}$). In recent years researchers have judiciously focused on the seroconversion and exact age of vaccination. These studies have concluded that almost two third proportion of individuals developed an adequate titer when vaccinated between 42^{nd} and 56^{th} day after birth. As animals continued to grow subsequent vaccination shots caused for a rise in antibody response. However, even at 12^{th} week of age around 10% puppies exhibited underwhelming antibody titers owing to the persistence of interfering maternal antibodies[67]. Immunization with vaccine is believed to be more efficacious as maternal antibody titers plummet to concentrations lower than 1:10

[68]. Despite presence of adequate scientific evidence regarding cross strain immunization, as different strains have emerged all across the world, efficacy of vaccines based on CPV type 2 strain has come under serious scrutiny [21, 22, 24, 28, 37]. However, recent studies have demonstrated that CPV-2 and CPV-2b based vaccines are adequate in developing protection against ever newer viral strains [69].

Recombinant Vaccine: Recombinant vaccines have been developed to elicit immunogenic responses against CPV in pups. Considering the fact that VP2 protein is integral to the antigenic presentation of CPV, it has been transgenically introduced into a baculovirus. VP2 protein produced by this recombinant virus was structurally and immunologically similar to naturally produced VP2. When these virus-like particles were used to immunize animals, they have been found to illicit adequate anti-CPV response verified by monolayer protection assays, enzyme-linked immunosorbent assay and an assay for the inhibition of hemagglutination. A dose of ca. 10 micrograms of recombinant VP2 brought about a reasonable protective response [70].

DNA Vaccine: DNA vaccines have been developed quite some time ago but they are still in experimental stages. They have been rigorously tested against virulent canine parvovirus and results observed were quite promising [71].

Peptide Vaccine: Peptide based CPV vaccines are being tested in rabbits whereby peptides similar to VP2 capsid proteins were synthesized and subsequently used for inoculation. Researchers have identified 23 residues of VP2 within the N-terminal capable of eliciting antibody response. In case of a synthetic peptide vaccine, orientation of the peptides have been deemed quite important [72].

Therapy: Most animals suffering from the clinical manifestation of CPV die of shock developing as a consequence of dehydration. Therefore, adequate fluid and electrolyte infusion are paramount for animal's survival [5]. Employing broad spectrum antibiotics namely; ampicillin, erythromycin, chloramphenicol, gentamycin has been found efficaciousness in avoiding secondary bacterial infections while the animal is in moribund state. In some cases when animals developed severe hemorrhagic gastroenteritis, Norfloxacin and nalidixic acid were proven to mitigate a several disease symptoms [20]. In acute cases where animals started to develop hypovolemic or cardiogenic shock, administration of short acting steroids has also been effective as a life saving measure. Severe diarrhea, being a pathognomonic lesion of this disease causes severe losses in serum bicarbonate and potassium levels. These electrolytes must be replenished along with fluid replacement therapy as to avoid metabolic acidosis. Any oral intake induces vomiting so animals must be kept off-feed until they recover [62]. Administration of a hyperimmune serum has been found efficacious in early phases of the disease and has exhibited reduction in mortalities associated with this disease [73]. Retching and vomiting could be controlled by parenteral administration of metoclopramide or chlorpromazine at 0.5 mg/kg body weight after every 8 h intervals while anti-histamines namely, ranitidine, cimetidine and famotidine can also be administered to check gastric problems [39].

Prevention and Control: Canine parvovirus is extremely hardy in the environment and able to tolerate temperature extremes. Disinfectants and antiseptics are not very effective against it either. Dogs infected with the virus shed 35 million viral particles per ounce of their stool for up to two weeks post exposure [41]. Virus loses its infectivity Indoors, 1 month after contamination. However viral persistence in outdoor setting varies as per sunlight exposure, whereby shaded areas remain contaminated for about 7 months while open aerated spaces clear up in 5. A 30 parts water and 1 part bleach concoction is considered an effective decontaminant on marbled or tiled floors against this virus [41]. While patches of dirt may require irrigation and subsequent drying. a pesticide sprayer may be employed to disperse Potassium per oxymonosulfate which has relatively good activity against contaminated organic matter [74].

Expected Benefits of the Review: This review of literature has been a comprehensive study regarding current perspectives in seroprevalence, diagnostics and therapeutics in context of veterinary practice in Pakistan. The study has aimed to abridge information regarding strain variability of CPV in indigenous and foreign settings. It would be a concise evaluation of recommended practices for diagnosis, therapy and immunization serving interests of small animal practitioners in developing countries.

CONCLUSION

In summary, Canine parvovirus is extremely virulent and contagious virus that infects young dogs. Due to its sapronotic nature, it is not possible to completely eliminate viral presence from home or kennel. If proper regiments and cold chain maintenance are employed, modified live vaccines are safe for use. However, due to interaction with maternal antibodies and the subsequent gap in vaccine coverage all puppies experience a window of susceptibility during which they are at high risk of contracting this disease. As viral mutations continue, the host range may expand leading to the emergence of deadly outbreaks. Vaccines available in the market have indeed provided adequate immunization against all existing varieties of CPV but this situation may rapidly change if resolute steps are not taken for collection, evaluation and immunization against newer strains namely CPV-2c. Therefore, it is believed that we must not rely on the cross-strain coverage of available vaccines but invest in developing homologous vaccines based on current or newer variants employing nanoparticle delivery mechanisms [75]. Transmission of these viral strains to sylvatic communities should be avoided by implementing stringent sanitary procedures at zoos. Despite the availability of live attenuated and inactivated parvovirus vaccines in Pakistan, dogs usually are not vaccinated against the disease. A large number of stray dog populations also act as incubators for the diseases, thereby maintaining constant endemicity [76]. Several puppies are regularly diagnosed with the said disease using HI, HA, PCR or ELISA.

Molecular epidemiology and seroprevalence of endemic CPV strains must be extensively investigated to avoid any catastrophic outbreaks. Spread of disease must be impeded by implementing widescale preventive measures and mass immunizations. Patient survivability improves several folds by adequate fluid replenishing therapeutic measures and symptomatic treatments involving use of antibiotics, anti-emetics and electrolytes.

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REFERENCES

1. Appel, M.J., F.W. Scott and L.E. Carmichael, 1979. Isolation and immunisation studies of a canine parco-like virus from dogs with haemorrhagic enteritis. The Veterinary Record, 105(8): 156-159.
2. Nho, W.G., J.H. Sur, A.R. Doster and S.B. Kim, 1997. Detection of canine parvovirus in naturally infected dogs with enteritis and myocarditis by in situ hybridization. Journal of Veterinary Diagnostic Investigation, 9(3): 255-260.
3. Shite, A., T. Guadu and B. Admassu, 2015. Challenges of rabies. International Journal of Basic and Applied Virology, 4(2): 41-52.
4. Buonavoglia, C., V. Martella, A. Pratelli, M. Tempesta, A. Cavalli, D. Buonavoglia, G. Bozzo, G. Elia, N. Decaro and L. Carmichael, 2001. Evidence for evolution of canine parvovirus type 2 in Italy. Journal of General Virology, 82(12): 3021-3025.
5. Nandi, S. and M. Kumar, 2010. Canine parvovirus: current perspective. Indian Journal of Virology, 21(1): 31-44.
6. Zaher, K.S., 2018. Parvoviruses in Dogs. International Journal of Basic and Applied Virology, 7(1): 01-06.
7. Tratschin, J., G.K. McMaster, G. Kronauer and G. Siegl, 1982. Canine parvovirus: relationship to wild-type and vaccine strains of feline panleukopenia virus and mink enteritis virus. Journal of General Virology, 61(1): 33-41.
8. Parker, J.S.L., W.J. Murphy, D. Wang, S.J. O'Brien and C.R. Parrish, 2001. Canine and feline parvoviruses can use human or feline transferrin receptors to bind, enter and infect cells. Journal of Virology, 75(8): 3896-3902.
9. Shackelton, L.A., C.R. Parrish, U. Truyen and E.C. Holmes, 2005. High rate of viral evolution associated with the emergence of carnivore parvovirus. Proceedings of the National Academy of Sciences, 102(2): 379-384.
10. Cavalli, A., M. Marinaro, C. Desario, M. Corrente, M. Camero and C. Buonavoglia, 2018. *In vitro* virucidal activity of sodium hypochlorite against canine parvovirus type 2. Epidemiology & Infection, 146(15): 2010-2013.

11. Truyen, U., A. Gruenberg, S.F. Chang, B. Obermaier, P. Veijalainen and C.R. Parrish, 1995. Evolution of the feline-subgroup parvoviruses and the control of canine host range *in vivo*. Journal of Virology, 69(8): 4702-4710.
12. Parrish, C.R., C.F. Aquadro, M.L. Strassheim, J.F. Evermann, J.Y. Sgro and H.O. Mohammed, 1991. Rapid antigenic-type replacement and DNA sequence evolution of canine parvovirus. Journal of Virology, 65(12): 6544-6552.
13. Nandi, S., S. Chidri, M. Kumar and R.S. Chauhan, 2010. Occurrence of canine parvovirus type 2c in the dogs with haemorrhagic enteritis in India. Research in Veterinary Science, 88(1): 169-171.
14. Decaro, N., V. Martella, C. Desario, A.L. Bellacicco, M. Camero, L. Manna, D. D'Aloja and C. Buonavoglia, 2006. First detection of canine parvovirus type 2c in pups with haemorrhagic enteritis in Spain. Journal of Veterinary Medicine, Series B, 53(10): 468-472.
15. Nakamura, M., Y. Tohya, T. Miyazawa, M. Mochizuki, H.T.T. Phung, N.H. Nguyen, L.M.T. Huynh, L.T. Nguyen, P.N. Nguyen and P. V. Nguyen, 2004. A novel antigenic variant of canine parvovirus from a Vietnamese dog. Archives of Virology, 149(11): 2261-2269.
16. Nandi, S., R. Anbuzhagan and M. Kumar, 2010. Strain differentiation and characterization of canine parvovirus by PCR and RE mapping. Indian Journal of Biotechnology, 9(1): 38-42.
17. Steinel, A., C.R. Parrish, M.E. Bloom and U. Truyen, 2001. Parvovirus infections in wild carnivores. Journal of Wildlife Diseases, 37(3): 594-607.
18. Ogbu, K.I., B.M. Anene, N.E. Nweze, J.I. Okoro, M.M. Danladi and S.O. Ochai, 2017. Canine Parvovirus: A Review. International Journal of Science and Applied Research, 2(2): 74-95.
19. Houston, D.M., C.S. Ribble and L.L. Head, 1996. Risk factors associated with parvovirus enteritis in dogs: 283 cases (1982-1991). Journal of the American Veterinary Medical Association, 208(4): 542-546.
20. Jacobs, R.M., M.G. Weiser, R.L. Hall and J.J. Kowalski, 1980. Clinicopathologic features of canine parvoviral enteritis. Journal American Animal Hospital Association, 16: 809-814.
21. De la Torre, D., E. Mafla, B. Puga, L. Erazo, C. Astolfi-Ferreira and A.P. Ferreira, 2018. Molecular characterization of canine parvovirus variants (CPV-2a, CPV-2b and CPV-2c) based on the VP2 gene in affected domestic dogs in Ecuador. Veterinary World, 11(4): 480.
22. Clark, N.J., J.M. Seddon, M. Kyaw-Tanner, J. Al-Alawneh, G. Harper, P. McDonagh and J. Meers, 2018. Emergence of canine parvovirus subtype 2b (CPV-2b) infections in Australian dogs. Infection, Genetics and Evolution, 58: 50-55.
23. Gogone, I.C.V.P., F.R.O. De Barros, F. Possatti, A.A. Alfieri and E. Takiuchi, 2020. Detection of canine parvovirus types 2b and 2c in canine faecal samples contaminating urban thoroughfares in Brazil. Canadian Journal of Microbiology, 66(2): 138-143.
24. Polat, P.F., A. Şahan, G. Aksoy, M.O. Timurkan and E. Dinçer, 2019. Molecular and restriction fragment length polymorphism analysis of canine parvovirus 2 (CPV-2) in dogs in southeast Anatolia, Turkey. Onderstepoort Journal of Veterinary Research, 86(1): 1-8.
25. Calatayud, O., F. Esperón, R. Velarde, Á. Oleaga, L. Llaneza, A. Ribas, N. Negre, A. De La Torre, A. Rodríguez and J. Millán, 2020. Genetic characterization of Carnivore Parvoviruses in Spanish wildlife reveals domestic dog and cat-related sequences. Transboundary and Emerging Diseases, 67(2): 626-634.
26. Charoenkul, K., R. Tangwangvivat, T. Janetanakit, S. Boonyapisitsopa, N. Bunpapong, S. Chaiyawong and A. Amonsin, 2019. Emergence of canine parvovirus type 2c in domestic dogs and cats from Thailand. Transboundary and Emerging Diseases, 66(4): 1518-1528.
27. Duque-García, Y., M. Echeverri-Zuluaga, J. Trejos-Suarez and J. Ruiz-Saenz, 2017. Prevalence and molecular epidemiology of Canine parvovirus 2 in diarrheic dogs in Colombia, South America: A possible new CPV-2a is emerging? Veterinary Microbiology, 201: 56-61.
28. Brewin, J., E. Graham, J. Daly and S. Dunham, 2017. Characterisation of canine parvovirus (CPV-2) circulating in the UK, In BSAVA Congr. Proc. 2017, BSAVA Library, pp: 552.
29. Jafri, S.A. and M. Rabbani, 1999. Prevalence of canine diseases in Lahore area. Pakistan Veterinary Journal, 19(1): 40-42.
30. Towakal, F., M. Rabbani, K. Muhammad, M.S. Khan and M.Z. Shabbir, 2010. Major strains of canine parvovirus present in dog population of Pakistan. Pakistan Journal of Zoology, 42(6): 833-836.
31. Umar, S., A. Ali, M. Younus, M.K. Maan, A. Shahzad, W.A. Khan and M. Irfan, 2015. Prevalence of canine parvovirus infection at different pet clinics in Lahore, Pakistan. Pakistan Journal of Zoology, 47(3): 657-663.

32. Shabbir, M.Z., M.U. Sohail, U.N. Chaudhary, W. Yaqub, I. Rashid, M.H. Saleem and M. Munir, 2017. Genetic characterization of canine parvovirus from dogs in Pakistan. Acta Virol, 61(2): 175-182.
33. Kelman, M., L. Harriott, M. Carrai, E. Kwan, M.P. Ward and V.R. Barrs, 2020. Phylogenetic and geospatial evidence of canine parvovirus transmission between wild dogs and domestic dogs at the urban fringe in Australia. Viruses, 12(6): 663.
34. Horecka, K., N. Ratnayaka and E.A. Davis, 2020. Changes in Mass Treatment of the Canine Parvovirus ICU Population in Relation to Public Policy Changes during the COVID-19 Pandemic. Viruses, 12(12): 1419.
35. Agnihotri, D., Y. Singh, S. Maan, V. Jain and A. Kumar, 2017. Molecular detection and clinico-haematological study of viral gastroenteritis in dogs. Haryana Veterinarian, 56(1): 72-76.
36. Salem, N.Y., 2014. Canine viral diarrhea: clinical, hematologic and biochemical alterations with particular reference to in-clinic rapid diagnosis. Global Veterinaria, 13(3): 302-307.
37. Franzo, G., C.M. Tucciarone, S. Casagrande, M. Caldin, M. Cortey, T. Furlanello, M. Legnardi, M. Cecchinato and M. Drigo, 2019. Canine parvovirus (CPV) phylogeny is associated with disease severity. Scientific Reports, 9(1): 1-8.
38. Bhargavi, M., B. Shobhamani, K.N. Kumari and C. Srilatha, 2017. Therapeutic management of dogs affected with canine parvo virus (CPV) infection. International Journal of Environmental Science and Technology, 6(5): 2797-2803.
39. Khatri, R., M.H. Poonam and P.C.S. Minakshi, 2017. Epidemiology, pathogenesis, diagnosis and treatment of canine parvovirus disease in dogs: A mini review abstract. Journal of Veterinary Science & Medical Diagnosis, 6(3): 2.
40. Robinson, W.F., C.R. Huxtable and D.A. Pass, 1980. Canine parvoviral myocarditis: a morphologic description of the natural disease. Veterinary Pathology, 17(3): 282-293.
41. Nandi, S., G.K. Sharma, V. Gupta, P. Deol and V. Chander, 2019. Global Scenario of Canine Parvovirus Mutants: Epidemiology, Diagnostics and Immunoprophylactic Agents. JSM Veterinary Medicine and Research, 2: 12.
42. Sinani, A. and I. Kusi, 2016. Pathologic Findings in Dogs Died of CPV-2 in Kosovo. Albanian Journal of Agricultural Sciences, 15(3): 154.
43. Ford, J., L. McEndaffer, R. Renshaw, A. Molesan and K. Kelly, 2017. Parvovirus infection is associated with myocarditis and myocardial fibrosis in young dogs. Veterinary Pathology, 54(6): 964-971.
44. Fagbohun, O.A., T.A. Jarikre, O.O. Alaka, R.D. Adesina, O.O. Ola, M. Afolabi, O.A. Oridupa, T.O. Omobowale and B.O. Emikpe, 2020. Pathology and molecular diagnosis of canine parvoviral enteritis in Nigeria: case report. Comparative Clinical Pathology, 29: 887-893.
45. Behera, S.K., Y.D. Singh, P. Roychoudhury, R.S. Arya, P. Behera, M.A. Ali, H. Prasad, K. Sarma, J.B. Rajesh and C. GE, 2020. Clinico-pathological and necropsy findings in a 4-month old mixed-breed pup with canine parvovirus-2 infection and its genetic characterization. Journal of Entomology and Zoology Studies, 8(5): 573-577.
46. Li, C., J. Tang, Z. Chen, G. Niu and G. Liu, 2019. A divergent canine parvovirus type 2c (CPV-2c) isolate circulating in China. Infection, Genetics and Evolution, 73: 242-247.
47. Kang, J.I., N.Y. Park and H.S. Cho, 2006. Detection of canine parvovirus in fecal samples using loop-mediated isothermal amplification. Journal of Veterinary Diagnostic Investigation, 18(1): 81-84.
48. Hoang, M., H. Wu, Y. Lien, M. Chiou and C. Lin, 2019. A SimpleProbe® real-time PCR assay for differentiating the canine parvovirus type 2 genotype. Journal of Clinical Laboratory Analysis, 33(1): e22654.
49. Osterhaus, A., G. Van Steenis and P. De Kreek, 1980. Isolation of a virus closely related to feline panleukopenia virus from dogs with diarrhea. Zentralblatt Für Veterinärmedizin Reihe B, 27(1): 11-21.
50. Mohan, R., D.C. Nauriyal and K.B. Singh, 1993. Detection of canine parvo virus in faeces, using a parvo virus ELISA test kit. Indian Veterinary Journal (India), 70(4): 301-303.
51. Singh Dahiya, S. and D.D. Kulkarni, 2004. Optimization of haemagglutination test for the detection of canine Parvovirus infection. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases, 25(2): 119-120.
52. Shadfar, S., A. Shabestari, M.B. Zendeh, B. Gasemi and S.H. Zamzam, 2012. Evaluation of Toxoplasma Gondii IgG antibodies in stray and household dogs by ELISA. Global Veterinaria, 9(1): 117-122.

53. Litster, A.L., B. Pressler, A. Volpe and E. Dubovi, 2012. Accuracy of a point-of-care ELISA test kit for predicting the presence of protective canine parvovirus and canine distemper virus antibody concentrations in dogs. The Veterinary Journal, 193(2): 363-366.
54. Proksch, A.L., S. Unterer, S. Speck, U. Truyen and K. Hartmann, 2015. Influence of clinical and laboratory variables on faecal antigen ELISA results in dogs with canine parvovirus infection. The Veterinary Journal, 204(3): 304-308.
55. Burtonboy, G., F. Coignoul, N. Delferriere and P.P. Pastoret, 1979. Canine hemorrhagic enteritis: detection of viral particles by electron microscopy. Archives of Virology, 61(1): 1-11.
56. Zhao, Y., A. Kolliopoulou, F. Ren, Q. Lu, V. Labropoulou, L. Swevers and J. Sun, 2019. Transcriptional response of immune-related genes after endogenous expression of VP1 and exogenous exposure to VP1-based VLPs and CPV virions in lepidopteran cell lines. Molecular Genetics and Genomics, 294(4): 887-899.
57. Carmichael, L.E., J.C. Joubert and R.V. Pollock, 1980. Hemagglutination by canine parvovirus: serologic studies and diagnostic applications. American Journal of Veterinary Research, 41(5): 784-791.
58. Kumar, M., S. Chidri and S. Nandi, 2010. Molecular cloning and restriction endonuclease analysis of canine parvovirus DNA amplified by polymerase chain reaction. Global Veterinaria, 4(2): 125-129.
59. Hirasawa, T., T. Kaneshige and K. Mikazuki, 1994. Sensitive detection of canine parvovirus DNA by the nested polymerase chain reaction. Veterinary Microbiology, 41(1-2): 135-145.
60. Kumar, A., J.S. Dharmadheeran, A. Kumar and S.S. Thakral, 2004. Strain Identification and Characterization of Vp I and Vp II Gene of Canine Parvovirus of Indian Origin. Journal of Applied Animal Research, 25(1): 57-60.
61. Decaro, N., G. Elia, V. Martella, M. Campolo, C. Desario, M. Camero, F. Cirone, E. Lorusso, M.S. Lucente and D. Narcisi, 2006. Characterisation of the canine parvovirus type 2 variants using minor groove binder probe technology. Journal of Virological Methods, 133(1): 92-99.
62. Singh, M., V. Chander and S. Nandi, 2019. Canine Parvovirus, In Recent Adv. Anim. Virol., Y.S. Malik, R.K. Singh, M.P. Yadav, eds., 1st ed., Springer, pp: 207-233.
63. Nandi, S., M. Kumar, T.K. Mohapatra and C. Ravishankar, 2013. Emergence of canine parvovirus-2 variants and its impact on vaccination. World Applied Sciences Journal, 23(10): 1366-1376.
64. Kara, A., 2020. Questions on Immunization and Vaccination and Short Answers. Cocuk Enfeksiyon Dergisi, 14(3): E155-E156.
65. Thomas, J., M. Singh, T.K. Goswami, P. Glora, S. Chakravarti, V. Chander, V. Upmanyu, S. Verma, C. Sharma and K. Mahendran, 2017. Determination of immune status in dogs against CPV-2 by recombinant protein based latex agglutination test. Biologicals, 49: 51-56.
66. Yohannes, S., B.G. Michael and W. Yadeta, 2020. Veterinary Vaccines Handling, Transportation and Storage: Factors Challenging their Efficacy and Their Adverse Effects to the Host. Global Veterinaria, 22(3): 121-127.
67. Truyen, U., 2006. Evolution of canine parvovirus-a need for new vaccines? Veterinary Microbiology, 117(1): 9-13.
68. Schultz, R.D., 2006. Duration of immunity for canine and feline vaccines: a review. Veterinary Microbiology, 117(1): 75-79.
69. Larson, L.J. and R.D. Schultz, 2008. Do two current canine parvovirus type 2 and 2b vaccines provide protection against the new type 2c variant? Veterinary Therapeutics, 9(2): 94.
70. De Turiso, J.A.L., E. Cortes, C. Martinez, R.R. De Ybanez, I. Simarro, C. Vela and I. Casal, 1992. Recombinant vaccine for canine parvovirus in dogs. Journal of Virology, 66(5): 2748-2753.
71. Gupta, P.K., A. Rai, N. Rai, A.A. Raut and S. Chauhan, 2005. Cloning of canine parvovirus VP2 gene and its use as DNA vaccine in dogs. Current Science, 88(5): 778-782.
72. Hasan, K., D. Rathnamma, H.D. Narayanaswamy, V. Malathi, N. Tomar, S. Gupta and S. V Singh, 2017. Current scenario and future perspectives of CPV-2 vaccines in India. Adv. Anim. Vet. Sci., 5(11): 446-448.
73. Suartini, G.A.A., A. Suprayogi, W.T. Wibawan, I. Sendow and G.N. Mahardika, 2014. Intravenous administration of chicken immunoglobulin has a curative effect in experimental infection of canine parvovirus. Global Veterinaria, 13(5): 801-808.
74. Scott, F.W., 1980. Virucidal disinfectants and feline viruses. American Journal of Veterinary Research, 41(3): 410-414.

75. Hanafi, E.M., W.M. Ahmed, M.M. Zaabal, A.A. El-Hadi, H.H. El Khadrawy and E.A. Ghazy, 2013. An overview on applications of nanoparticles in biological systems. Global Journal of Pharmacology, 7(3): 348-359.
76. Asmare, K. and S. Mekuria, 2013. Gastrointestinal helminthes in dogs and community perception on parasite zoonosis at Hawassa city, Ethiopia. Global Veterinaria, 11(4): 432-440.
77. Decaro, N., C. Desario, D.D. Addie, V. Martella, M.J. Vieira, G. Elia, A. Zicola, C. Davis, G. Thompson and E. Thiry, 2007. Molecular epidemiology of canine parvovirus, Europe. Emerging Infectious Diseases, 13(8): 1222.
78. McElligott, S., P.J. Collins, R.D. Sleator, V. Martella, N. Decaro, C. Buonavoglia and H. O'Shea, 2011. Detection and genetic characterization of canine parvoviruses and coronaviruses in southern Ireland. Archives of Virology, 156(3): 495-503.
79. Decaro, N., C. Desario, F. Amorisco, M. Losurdo, G. Elia, A. Parisi, G. Ventrella, V. Martella and C. Buonavoglia, 2013. Detection of a canine parvovirus type 2c with a non-coding mutation and its implications for molecular characterisation. The Veterinary Journal, 196(3): 555-557.
80. Steinel, A., L. Munson, M. Van Vuuren and U. Truyen, 2000. Genetic characterization of feline parvovirus sequences from various carnivores. Microbiology, 81(2): 345-350.
81. Decaro, N., C. Desario, M. Billi, V. Mari, G. Elia, A. Cavalli, V. Martella and C. Buonavoglia, 2011. Western European epidemiological survey for parvovirus and coronavirus infections in dogs. The Veterinary Journal, 187(2): 195-199.
82. Miranda, C. and G. Thompson, 2016. Canine parvovirus: the worldwide occurrence of antigenic variants. Journal of General Virology, 97(9): 2043-2057.
83. Ntafis, V., V. Mari, N. Decaro, M. Papanastassopoulou, N. Papaioannou, R. Mpatziou, C. Buonavoglia and E. Xylouri, 2011. Isolation, tissue distribution and molecular characterization of two recombinant canine coronavirus strains. Veterinary Microbiology, 151(3-4): 238-244.
84. Majer-Dziedzic, B., A. Jakubczak and J. Zietek, 2011. Phylogenetic analysis of canine parvovirus CPV-2 strains and its variants isolated in Poland. Polish Journal of Veterinary Sciences, 14(3): 379-84.
85. Sutton, D., C. Vinberg, A. Gustafsson, J. Pearce and N. Greenwood, 2013. Canine parvovirus type 2c identified from an outbreak of severe gastroenteritis in a litter in Sweden. Acta Veterinaria Scandinavica, 55(1): 1-5.
86. Decaro, N. and C. Buonavoglia, 2012. Canine parvovirus-a review of epidemiological and diagnostic aspects, with emphasis on type 2c. Veterinary Microbiology, 155(1): 1-12.
87. Filipov, C., C. Desario, O. Patouchas, P. Eftimov, G. Gruichev, V. Manov, G. Filipov, C. Buonavoglia and N. Decaro, 2016. A ten-year molecular survey on parvoviruses infecting carnivores in Bulgaria. Transboundary and Emerging Diseases, 63(4): 460-464.
88. Demeter, Z., E.A. Palade, T. Soós, A. Farsang, C. Jakab and M. Rusvai, 2010. Misleading results of the Mbo II-based identification of type 2a canine parvovirus strains from Hungary reacting as type 2c strains. Virus Genes, 41(1): 37-42.
89. Cavalli, A., C. Desario, I. Kusi, V. Mari, E. Lorusso, F. Cirone, I. Kumbe, M.L. Colaianni, D. Buonavoglia and N. Decaro, 2014. Detection and genetic characterization of Canine parvovirus and Canine coronavirus strains circulating in district of Tirana in Albania. Journal of Veterinary Diagnostic Investigation, 26(4): 563-566.
90. Timurkan, M. and T. Oğuzoğlu, 2015. Molecular characterization of canine parvovirus (CPV) infection in dogs in Turkey. Veterinaria Italiana, 51(1): 39-44.
91. Chausov, E.V., V.A. Ternovoi, E.V. Protopopova, A.G. Durymanov, A.M. Shestopalov, V.B. Loktev and S.V. Netesov, 2011. Canine parvovirus strain Laika-1993, complete genome. GenBank: Accession Number JN033694.
92. Ahmed, A.F., 2012. Detection of Canine Parvovirus in Baghdad city by PCR technique. The Iraqi Journal of Veterinary Medicine, 36(0E): 95-98.
93. Fatima, U., A. Mehboob, M. Abid and T. Yaqub, 2017. Molecular Characterization and Evolutionary Analysis of Canine Parvo Viruses in Dogs. Hosts and Viruses, 4(2): 33.
94. Phromnoi, S., K. Sirinarumitr and T. Sirinarumitr, 2010. Sequence analysis of VP2 gene of canine parvovirus isolates in Thailand. Virus Genes, 41(1): 23-29.
95. Chou, S.J., H.T. Lin, J.T. Wu, W.C. Yang and K.W. Chan, 2013. Genotyping of canine parvovirus type 2 VP2 gene in southern Taiwan in 2011. Taiwan Vet. J., 39(2): 81-92.

96. Yi, L., M. Tong, Y. Cheng, W. Song and S. Cheng, 2016. Phylogenetic Analysis of Canine Parvovirus VP 2 Gene in C hina. Transboundary and Emerging Diseases, 63(2): e262-e269.
97. Yoon, S.H., W. Jeong, H.J. Kim and D.J. An, 2009. Molecular insights into the phylogeny of canine parvovirus 2 (CPV-2) with emphasis on Korean isolates: a Bayesian approach. Archives of Virology, 154(8): 1353-1360.
98. Soma, T., S. Taharaguchi, T. Ohinata, H. Ishii and M. Hara, 2013. Analysis of the VP2 protein gene of canine parvovirus strains from affected dogs in Japan. Research in Veterinary Science, 94(2): 368-371.
99. Dogonyaro, B.B., A.M. Bosman, K.P. Sibeko, E.H. Venter and M. van Vuuren, 2013. Genetic analysis of the VP2-encoding gene of canine parvovirus strains from Africa. Veterinary Microbiology, 165(3-4): 460-465.
100. Steinel, A., C.R. Parrish, U. Truyen, M. Van Vuuren and E.H. Venter, 1998. Antigenic and genetic analysis of canine parvoviruses in southern Africa. Onderstepoort Journal of Veterinary Research, 65(4): 239-242.
101. Touihri, L., I. Bouzid, R. Daoud, C. Desario, A.F. El Goulli, N. Decaro, A. Ghorbel, C. Buonavoglia and C. Bahloul, 2009. Molecular characterization of canine parvovirus-2 variants circulating in Tunisia. Virus Genes, 38(2): 249-258.
102. Meers, J., M. Kyaw-Tanner, Z. Bensink and R. Zwijnenberg, 2007. Genetic analysis of canine parvovirus from dogs in Australia. Australian Veterinary Journal, 85(10): 392-396.
103. Ohneiser, S.A., S.F. Hills, N.J. Cave, D. Passmore and M. Dunowska, 2015. Canine parvoviruses in New Zealand form a monophyletic group distinct from the viruses circulating in other parts of the world. Veterinary Microbiology, 178(3-4): 190-200.
104. Hong, C., N. Decaro, C. Desario, P. Tanner, M.C. Pardo, S. Sanchez, C. Buonavoglia and J.T. Saliki, 2007. Occurrence of canine parvovirus type 2c in the United States. Journal of Veterinary Diagnostic Investigation, 19(5): 535-539.
105. Pedroza-Roldán, C., V. Páez-Magallan, C. Charles-Niño, D. Elizondo-Quiroga, R. Leonel De Cervantes-Mireles and M.A. López-Amezcua, 2015. Genotyping of Canine parvovirus in western Mexico. Journal of Veterinary Diagnostic Investigation, 27(1): 107-111.
106. Pinto, L.D., A.F. Streck, K.R. Gonçalves, C.K. Souza, Â.O. Corbellini, L.G. Corbellini and C.W. Canal, 2012. Typing of canine parvovirus strains circulating in Brazil between 2008 and 2010. Virus Research, 165(1): 29-33.
107. Pérez, R., P. Bianchi, L. Calleros, L. Francia, M. Hernández, L. Maya, Y. Panzera, K. Sosa and S. Zoller, 2012. Recent spreading of a divergent canine parvovirus type 2a (CPV-2a) strain in a CPV-2c homogenous population. Veterinary Microbiology, 155(2-4): 214-219.
108. Calderón, M.G., C. Romanutti, A. D'Antuono, L. Keller, N. Mattion and J. La Torre, 2011. Evolution of canine parvovirus in Argentina between years 2003 and 2010: CPV2c has become the predominant variant affecting the domestic dog population. Virus Research, 157(1): 106-110.
109. Aldaz, J., J. García-Díaz, L. Calleros, K. Sosa, G. Iraola, A. Marandino, M. Hernández, Y. Panzera and R. Pérez, 2013. High local genetic diversity of canine parvovirus from Ecuador. Veterinary Microbiology, 166(1-2): 214-219.